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Development of Antibodies to the Nonstructural Protein NS1 of Parvovirus B19 During Acute Symptomatic and Subclinical Infection in Pregnancy: Implications for Pathogenesis Doubtful

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At present little is known about the mechanisms influencing the course and severity of parvovirus B19 infection. Antibodies to the parvovirus nonstructural protein NS1 were reported in patients with parvovirus-associated arthritis and those with persisting infection but not in those without complications, suggesting a potential involvement of NS1 or anti-NS1 antibodies in pathogenesis. The immune response to NS1 was examined retrospectively in 33 pregnant women with acute parvovirus B19 infection, 14 of whom experienced symptomatic infection and 19 in whom the infection was subclinical. Antibodies to NS1 were found in 15 (45%) of the women, seven with symptomatic and eight with subclinical infection. No association was found between the development of anti-NS1 antibodies and the occurrence of fetal complications. Of the seven cases in which fetal complications were observed, anti-NS1 antibodies were detected in only three. The finding that an immune response to NS1 can also be demonstrated in patients with asymptomatic infection suggests that anti-NS1 antibodies do not appear to represent a marker for an altered or severe course of infection in pregnant women or to contribute significantly to pathogenesis. Since anti-NS1 antibodies first become detectable at least six weeks postinfection, their presence can be used to exclude acute infection in patients with unclear serology or be used to aid differential diagnosis of rashlike illnesses. ***J. Med. Virol. 56: 192–198, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: nonstructural protein; antibodies; parvovirus

INTRODUCTION

The most common manifestation of parvovirus B19 infection is erythema infectiosum, a mild febrile illness with rash which often occurs during childhood [Anderson et al., 1983]. In recent years the virus has been associated with a wide variety of disease syndromes, including transient aplastic anemia, arthritis and arthropathy, vasculitis, and hepatitis [Pattison et al., 1981; Reid et al., 1985; White et al., 1985; Brown et al., 1994; Finkel et al., 1994; Yoto et al., 1996]. During pregnancy, transmission of the virus to the fetus can lead to hydrops fetalis, spontaneous abortion, or intrauterine death [Public Health Laboratory Working Party of Fifth Disease, 1990; Schwarz et al., 1990]. However, in at least 20% of adults the infection is subclinical [Woolf et al., 1989]. Our own data suggest that as many as 65% of infected pregnant women experience no symptoms [Enders, 1998]. During pregnancy many infections are undiagnosed or come to light retrospectively following the occurrence of fetal complications. The mechanisms influencing the course and severity of parvovirus B19 infection are as yet poorly understood.

Recently, the immune response to the nonstructural protein of parvovirus B19 (NS1) has been investigated and there is a suggestion that anti-NS1 antibodies or the NS1 protein itself may be implicated in pathogenesis [Poblotzki et al., 1995a, 1995b]. Antibodies to NS1 were detected in three patients with parvovirus B19-associated arthritis but not in patients with acute or past infection without complications, which suggested a possible role for anti-NS1 antibodies in pathogenesis [Poblotzki et al., 1995a]. Anti-NS1 antibodies were also detected in three additional patients with persisting parvovirus infection and it was suggested that the appearance of NS1-specific antibodies might indicate an altered course of viral infection leading to the establishment of a persistently active infection [Poblotzki et al., 1995b].

The possible link between parvovirus B19 pathogen-

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TABLE I. Cases of Acute Parvovirus B19 Infection With Fetal Complications[a]

Patient	Maternal symptoms	Diagnosis of acute infection	Anti-NS1 IgG antibodies	Fetal symptoms	Prenatal diagnosis
13	none	wop 22	yes	wop 22, hydrops, ascites, pericardial effusion, anemia Hb 3.6	wop 22 fb +, f. IgM + wop 28 fb +, f. IgM + wop 29 af +
14	wop 23, joint pain	wop 24	yes	wop 26, mild pericardial effusion, mild ascites	wop 26 fb +, f. IgM +, af
15	wop 15, rash, flulike symptoms	wop 20	yes	wop 20, hydrops, ascites, pericardial effusion, anemia Hb 3.4	wop 20, fb +, f. IgM +
24	wop 8 fever, flulike symptoms	wop 8	no	wop 16, neck odema	n.d.
27	none	wop 22	no	wop 22, hydrops, anemia Hb 4.9	wop 22 fb +, f. IgM –, af
30	wop 20, rash	wop 21	no	wop 24, discrete pericardial effusion, mild anemia Hb 9.0	wop 24 fb*, f. IgM + wop 25 fb +, f. IgM +
33	none	wop 12	no	wop 24, hydrops, ascites	wop 24 fb +

[a]wop = week of pregnancy; fb + = fetal blood PCR-positive; f. IgM = fetal IgM; af = amniotic fluid; Hb = hemoglobin g/100 ml (normal values for wop 18–23, 11.7 ± 0.8; wop 24–29, 12.8 ± 1.1).

esis and the anti-NS1 response lead us to investigate the development of anti-NS1 antibodies in women with acute parvovirus B19 infection to establish whether there is an association between development of anti-NS1 antibodies and clinical symptoms, particularly since symptoms are reported only by a minority of women. We therefore examined sera from both symptomatic and subclinical acute parvovirus B19 infections during pregnancy. Furthermore, the possibility was also investigated of a link between the development of anti-NS1 antibodies and the occurrence of fetal complications or their possible influence on pregnancy outcome.

MATERIALS AND METHODS

Sera

A total of 133 sera from 33 women with acute parvovirus B19 infection during pregnancy were investigated. These sera were sent to the laboratory for diagnosis of acute parvovirus B19 infection between 1995 and 1997 from obstetricians and prenatal diagnostic centers throughout Germany. The women acquired parvovirus infection between the 7th and 32nd week of pregnancy, with the majority of women becoming infected during the second trimester. Clinical symptoms consistent with parvovirus B19 infection were reported by 14 of the 33 women. Rash was reported by 10, five had joint pain, three had flulike symptoms; in addition, lymphadenopathy and myalgia were reported in two cases. The remaining 19 women reported no symptoms. In such cases samples were received following known contact with parvovirus B19 infected individuals or retrospectively following occurrence of fetal complications. At least one follow-up sample was obtained from each woman. The interval between the first and last samples or between symptoms and last sample was at least two months in each case. Pregnancy outcome was documented by obtaining clinical information. Fetal complications were experienced by seven of the women, the details of which are presented in Table I. Thirty pregnancies proceeded to term; there was one spontaneous abortion in week 16 of pregnancy. One pregnancy was terminated in week 24 and in another case no follow-up information was received concerning pregnancy outcome.

Also included in the study were seropositive sera (IgG-positive, IgM-negative) from 25 pregnant women who had previous parvovirus B19 infection, 148 nonpregnant women (comprising 49 women aged 20 to 29, 57 women aged 30 to 39, and 42 women aged 50 to 59), and 58 blood donors.

As a control for the specificity of the anti-NS1 immunoblot assay, 128 sera from women who were seronegative for parvovirus B19 structural proteins were also included in the study.

Diagnosis of Acute Parvovirus B19 Infection

Acute parvovirus B19 infection in the 33 pregnant women was diagnosed serologically by the presence of IgM and rising IgG antibodies. There were nine seroconversions. In addition, in some cases parvovirus B19 DNA detection was carried out using PCR as described below. Three of the acute infections were serologically diagnosed retrospectively following the occurrence of fetal complications.

Serology

Enzyme Immunoassay (EIA). IgG and IgM antibodies to the structural parvovirus B19 capsid protein VP2 were determined using commercial EIA kits (Biotrin, Dublin, Ireland) in accordance with the manufacturer's instructions. Quantification of IgG antibodies in IU/ml was facilitated with reference to the international parvovirus standard serum for IgG as described previously [Searle et al., 1997]. IgG values of less than 2 IU/ml are considered negative, values of 2–5 IU/ml are equivocal, values of 6–15 IU/ml are weekly positive, and those of 16 or more are considered

positive. Samples that had titers greater than that of the international parvovirus B19 standard serum were not titrated further and were assigned a value of >100 IU/ml. IgM results were expressed as index values, whereby values of >1.0 are considered positive.

Immunoblot. IgG and IgM antibodies to the nonstructural protein NS1 were detected using an immunoblot assay from Mikrogen (Munich, Germany) in accordance with the manufacturer's instructions. The immunoblot assay employs recombinant parvovirus B19 antigens expressed in *Escherichia coli* and allows detection of antibodies to three fragments encompassing the structural antigens VP1 and VP2 as well as the nonstructural protein NS1.

Parvovirus B19 PCR

PCR was carried out by a single-round PCR amplification reaction, which coamplifies VP1 and VP2 sequences in a single step as described previously [Searle et al., 1998]. Semiquantitative evaluation of the viral load was performed by preparing serial dilutions of the specimens and a DNA preparation of a parvovirus B19 full-length clone of known concentration (10 fg of the clone represents approximately 100 B19 genome copies). The results of the semiquantitative PCR analysis were expressed as follows: $<10^3$ copies = negative; 10^3 to 10^4 copies = +; 10^4 to 10^5 copies = ++; and 10^5 to 10^6 copies = +++. All samples from an individual patient were tested in a single test run to ensure comparability of results.

RESULTS AND DISCUSSION

The specificity of the immunoblot assay for anti-NS1 antibodies was assessed by testing 128 sera from women who were seronegative for parvovirus structural proteins. No anti-NS1 reactivity was detected in any of these women (data not shown).

In order to determine whether there was an association between anti-NS1 antibodies and clinical symptoms, we retrospectively investigated the sera of 33 pregnant women with acute parvovirus B19 infection, 14 with and 19 without maternal symptoms. An anti-NS1 response could not be detected in the initial serum samples from any of the 33 women with acute parvovirus B19 infection. However, during the course of infection, anti-NS1–specific antibodies of the IgG class became detectable in 15 (45%) of the 33 women, in 7 of the 14 women with symptomatic infection and in 8 of the 19 women in whom the infection was subclinical. The data for the 15 women who developed anti-NS1 antibodies are shown in Table II. Table III shows the results for the initial and last serum samples from the 18 women in whom anti-NS1 antibodies were not found. The findings contrast with those of Poblotzki et al. [1995a], which found anti-NS1 antibodies exclusively in patients suffering from severe parvovirus B19-associated arthritis and not in those with acute or past infection without complications. The small number of sera investigated by Poblotzki et al. [1995a] may partly explain the discrepant findings. In their study, only seven cases of acute parvovirus B19 were investigated, three with severe arthritis and four with the typical symptoms of erythema infectiosum, whereby an anti-NS1 antibody response was found only in those cases with arthritis. Another explanation may lie in the difference in the sensitivity of the assays used; whereas we employed an immunoblot assay, Poblotzki et al. [1995a] used an enzyme-linked immunosorbent assay (ELISA).

In order to determine the time course of development of anti-NS1 antibodies, we analyzed serial samples from all 15 women who were anti-NS1 antibody-positive following acute infection, the results of which are shown in Table II. Anti-NS1 antibodies first become detectable approximately six weeks after the onset of acute infection. In some cases the development of antibodies may take even longer (e.g., patient 5). Samples from the patients with erythema infectiosum investigated in the study of Poblotzki et al. [1995a] may have been taken shortly after the onset of infection, which might offer an explanation for the lack of anti-NS1 antibodies. In the majority of cases in our study, anti-NS1 antibodies became detectable before IgM levels had declined completely.

Anti-NS1 antibodies were detected in 7 (30%) of the 25 pregnant women with previous infection, in 28 (19%) of the 148 nonpregnant seropositive women, and in 17 (29%) of the 58 seropositive blood donors (data not shown). The overall detection rate of approximately 22% in individuals with previous infection contrasts with the findings of Poblotzki et al. [1995a], which did not detect anti-NS1 antibodies in any of 14 patients tested with previous infection. Interestingly, we found that the percentage of NS1-positive women fell dramatically with increasing age. Whereas 30% of the seropositive women in the 20- to 29-year age group had anti-NS1 antibodies, 21% of the 30- to 39-year-olds and only 2% of the 50- to 59-year-olds were anti-NS1–positive. Thus, the titer of anti-NS1 antibodies appears to decline more rapidly than titers to the structural proteins VP1 and VP2.

Transmission of parvovirus B19 to the fetus poses a risk of hydrops fetalis. Although cases of spontaneous remission have been reported, severe cases of hydrops generally result in fetal death if not treated rapidly by intrauterine transfusion with erythrocyte concentrate [Fairley et al., 1995]. Hydrops fetalis of varying degree occurred in 7 of the 33 pregnancies studies here (patients 13, 14, 15, 24, 27, 30, and 33). The data for these seven women are presented in Table I. Five of the pregnancies went to term, with four of the fetuses having been treated by intrauterine therapy with erythrocyte concentrate. There were two cases of fetal loss: one spontaneous abortion and one induced termination. Prenatal diagnosis revealed fetal infection by detection of parvovirus B19 DNA in amniotic fluid or fetal blood in each of the six cases tested. Anti-NS1 antibodies were found in only 3 (43%) of the 7 cases in which fetal complications occurred (patients 13, 14, and 15) and in 12 (46%) of the 26 patients without fetal complications.

TABLE II. Development of Anti-NS1 Antibodies in Pregnant Women With Acute Parvovirus B19 Infection[a]

Patient	Maternal symptoms	Fetal complications	Week of pregnancy	Anti-VP2 IgG EIA	Anti-VP2 IgM EIA	Anti-NS1 IgG[b] blot
1	none	none	13	41	8.9	neg
			15	55	6.6	neg
			19	76	2.0	pos
			23	>100	<1.0	pos
2	wop 18, rash and joint pain	none	18	24	6.8	neg
			23	32	5.5	neg
			28	72	3.1	pos
			32	84	1.7	pos
			del.	>100	1.6	pos
			p.del.	92	<1.0	pos
3	wop 8, flulike symptoms	none	10	31	5.0	neg
			16	93	1.8	neg
			21	77	1.0	wpos
			28	>100	<1.0	pos
			del.	>100	<1.0	pos
4	wop 9, exanthem	none	9	13	8.9	neg
			10	53	9.0	neg
			15	46	3.8	pos
			del.	84	<1.0	pos
5	none	none	17	<2	<1.0	neg
			21	<2	<1.0	neg
			24	<2	1.4	neg
			26	7	7.4	neg
			30	46	3.5	neg
			del.	77	1.1	pos
6	none	none	28	<2	<1.0	neg
			32	31	5.4	neg
			37	93	1.6	pos
			del.	>100	<1.0	pos
7	wop 17, rash	none	17	<2	7.1	neg
			18	53	7.7	neg
			20	55	5.9	neg
			25	63	2.2	pos
			30	>100	<1.0	pos
			del.	79	<1.0	pos
8	none	none	18	56	2.5	neg
			20	38	1.5	neg
			24	50	<1.0	pos
9	none	none	30	58	2.8	neg
			del.	>100	<1.0	pos
10	none	none	32	50	6.1	neg
			del.	60	1.7	pos
11	none	none	26	99	7.6	neg
			del.	>100	1.0	pos
12	wop 10, rash	none	19	33	13.4	neg
			del.	84	3.0	pos
13	none	wop 22, hydrops	22	42	3.9	neg
			del.	63	2.4	pos
14	wop 23, joint pain	wop 26, mild pericardial effusion	24	57	6.9	wpos
			25	90	4.3	pos
			26	>100	2.8	pos
			del.	>100	<1.0	pos
15	wop 15, rash, flulike symptoms	wop 20, hydrops	20	83	<1.0	wpos
			23	73	<1.0	pos
			del.	76	<1.0	pos

[a]wop = week of pregnancy; del. = at delivery; p.del. = postdelivery; pos = positive; wpos = weakly positive; neg = negative.
[b]Only results for first and last serum samples are shown.

The potential association between persistence of virus and anti-NS1 antibody development leads us to investigate the development of anti-NS1 antibodies in association with serum virus load in six patients. Figure 1 shows the time course of antibody development and semiquantitative PCR results for three patients with maternal symptoms (patients 2, 7, and 30). Figure 2 shows the corresponding data for three patients with asymptomatic infection (patients 5, 21, and 29). Anti-NS1 antibodies could be found in patients in whom viral DNA persisted for five months or more (patients 2 and 5), but also in patient 7 in whom virus levels de-

TABLE III. Pregnant Women With Acute Parvovirus B19 Infection: Anti-NS 1–Negative[a]

Patient	Maternal symptoms	Fetal complications	Week of pregnancy	Anti-VP2 IgG EIA	Anti-VP2 IgM EIA	Anti-NS1 IgG[b] blot
16	none	none	19	68	1.2	neg
			25	88	1.1	neg
17	wop 27, joint pain	none	25	<2	<1.0	neg
			del.	>100	4.4	neg
18	wop 13, rash, arthralgia, myalgia	none	15	>100	9.1	neg
			23	>100	2.5	neg
19	wop 7, fever, rash, lymphadenopathy	none	9	>100	2.4	neg
			del.	>100	<1.0	neg
20	none	none	24	56	7.0	neg
			del.	>100	6.4	neg
21	none	none	15	<2	<1.0	neg
			del.	>100	1.0	neg
22	none	none	19	29	2.7	neg
			del.	17	<1.0	neg
23	wop 27, exanthem	none	23	54	5.7	neg
			del.	>100	<1.0	neg
24	wop 8, fever, flulike symptoms	wop 16, spontaneous abortion	8	<2	1.1	neg
			18	86	1.4	neg
25	none	none	19	83	6.7	neg
			del.	>100	<1.0	neg
26	none	none	22	97	3.0	neg
			del.	73	<1.0	neg
27	none	wop 22, hydrops, anemia	22	88	4.0	neg
			del.	46	3.2	neg
28	none	none	17	83	3.2	neg
			del.	76	<1.0	neg
29	none	none	13	10	7.0	neg
			del.	63	<1.0	neg
30	wop 20, rash	wop 24, discrete pericardial effusion, mild anemia	21	27	2.0	neg
			del.	54	<1.0	neg
31	wop 7, joint pain wop 12, rash	none	9	19	7.9	neg
			del.	>100	1.4	neg
32	none	none	20	53	7.3	neg
			del.	>100	3.1	neg
33	none	wop 24, hydrops, termination	12	6	3.6	neg
			24	>100	<1.0	neg

[a]wop = week of pregnancy; del. = at delivery; pos = positive; neg = negative.
[b]Only results for first and last serum samples are shown.

clined more rapidly. Other patients with detectable parvovirus B19 DNA for up to four months postinfection did not develop anti-NS1 antibodies (patients 30 and 29). Therefore, extended persistence of virus does not appear to be a prerequisite for anti-NS1 antibody development. However, the sensitivity of the PCR assay cannot exclude the presence of low-titer viremia and thus a prolonged antigenic stimulation in some cases.

Determination of anti-NS1 antibodies may prove useful as a supplementary assay in routine diagnostic testing of pregnant women for parvovirus B19. Since anti-NS1 antibodies first appear at least six weeks after infection, a positive result can be used to aid differential diagnosis in patients with rashlike illnesses. In addition, the assay may aid diagnosis in women with borderline or weakly positive IgM results, which are not uncommon during pregnancy and which are difficult to interpret, especially in the case of subclinical infection. However, the utility of the assay is limited by the fact that only approximately 30% of women of childbearing appear to develop anti-NS1 antibodies. The ability of only a particular subgroup of individuals to mount an immune response against NS1 may be explained by differences in the virus replication cycle in certain hosts or perhaps by histocompatibility restriction. It is of interest that IgM antibodies to NS1 were detectable in only one of the patients investigated in our study (data not shown).

In conclusion, the development of anti-NS1 antibodies is not limited to persistently infected patients or to those with B19-associated arthritis. We also found anti-NS1 antibodies in two patients with life-threatening parvovirus B19-associated myocarditis (data not shown), which we reported on recently [Enders et al., 1998]. However, we have now established that an immune response to NS1 can also be demonstrated in patients with asymptomatic parvovirus B19

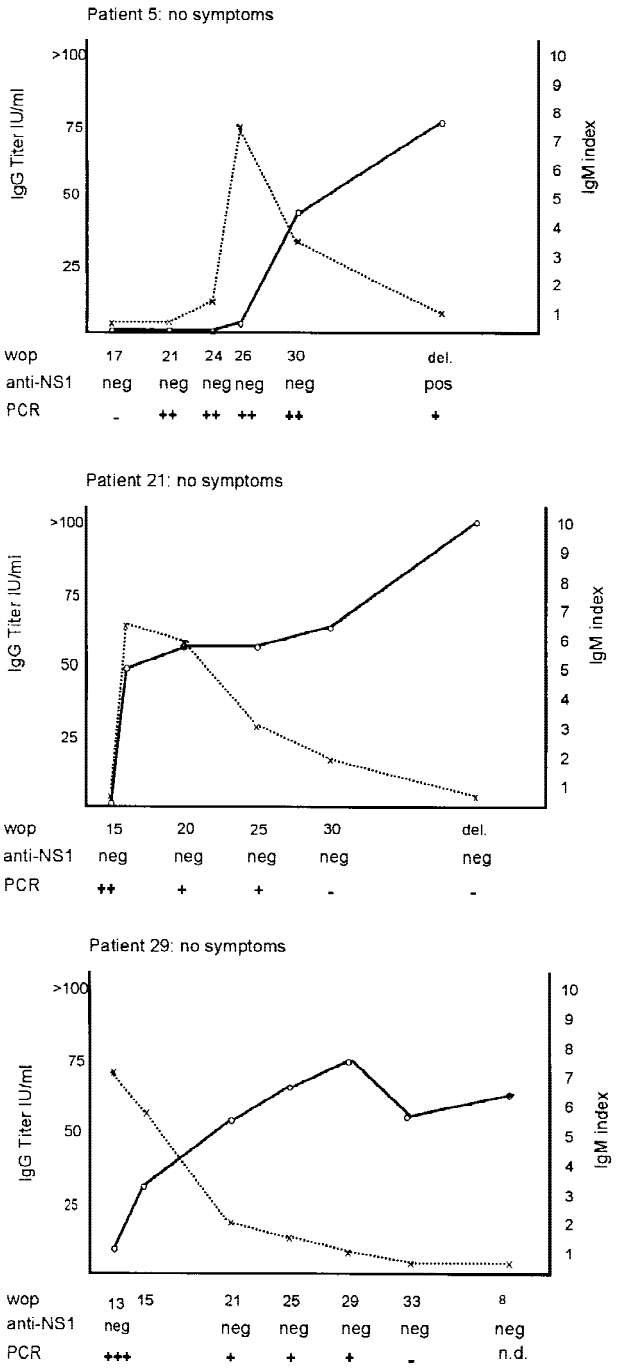

Fig. 1. Antibody development with respect to semiquantitative PCR results, symptomatic infection IgG (continuous line) and IgM (dashed line). Semiquantitative PCR: – denotes $<10^3$; +, 10^3 to 10^4; ++, 10^4 to 10^5; +++, 10^5 to 10^6. wop = week of pregnancy; del. = at delivery.

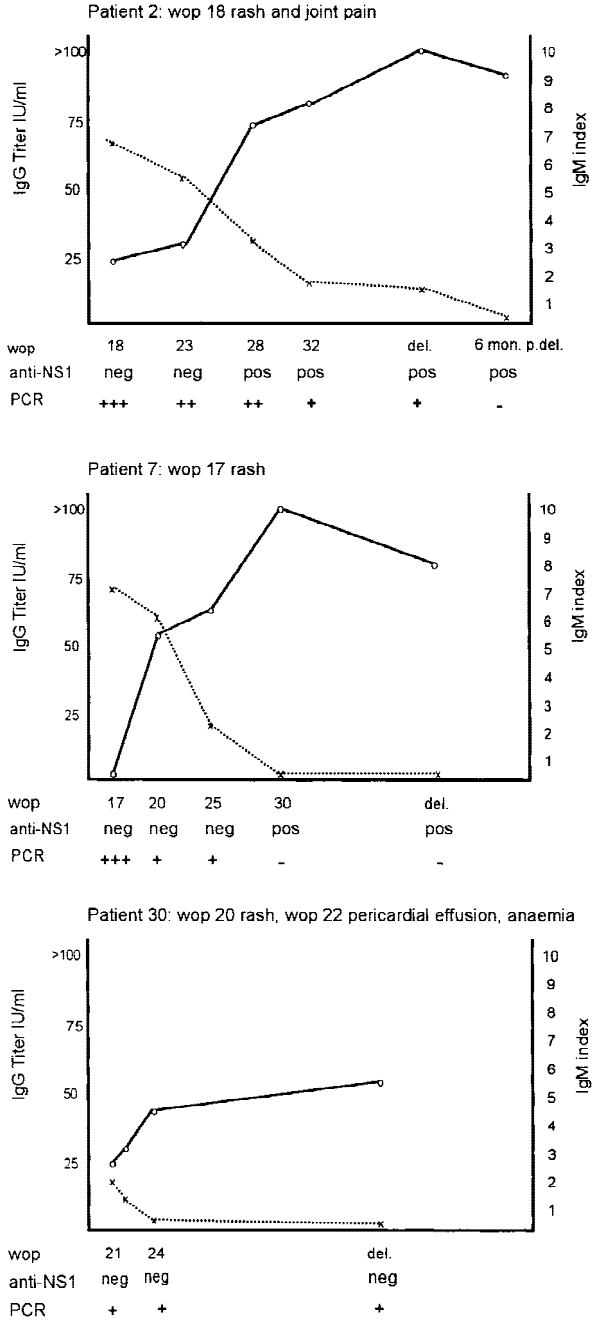

Fig. 2. Antibody development with respect to semiquantitative PCR results, subclinical infection IgG (continuous line) and IgM (dashed line). Semiquantitative PCR: – denotes $<10^3$; +, 10^3 to 10^4; ++, 10^4 to 10^5; +++, 10^5 to 10^6. wop = week of pregnancy; del. = at delivery; p. del. = postdelivery.

infection. The development of anti-NS1 antibodies does not, therefore, appear to represent a marker for an altered or severe course of infection or to contribute directly to pathogenesis. Further study is required to elucidate the mechanisms influencing the course and severity of parvovirus B19 infection, whereby both viral and host factors are likely to play a role.

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